sodium salt, as such or prepared either from the bis-cyclohexylammonium salt or from a barium salt). The stimulatory effect of fructose 1-phosphate on the activity of glycogen synthase *b* [2] was also independent of the type of cation (results not shown). In contrast, the effect of 20 mM-fructose 1-phosphate on the activation of glycogen synthase was highly dependent on the type of cation (Figs. 1*c*–1*f*); only the bis-cyclohexylammonium salt allowed synthase phosphatase to be moderately active in the presence of phosphorylase *a*; all the other preparations merely prolonged the latency, as expected from the slower inactivation of phosphorylase. It is noteworthy that 20 mM-cyclohexylamine inhibited the inactivation of phosphorylase without 'deinhibiting' the activation of glycogen synthase (Fig. 1*b*). In another experiment the bis-cyclohexylammonium salt was transformed into a potassium salt, which also behaved like the sodium salt (results not shown). We have not further investigated the mechanism of the discrepant effect of the bis-cyclohexylammonium salt of fructose 1-phosphate, but we checked that its stability in the gel-filtered extract was similar to that of the commercial sodium salt (less than 5% disappearance after a 20 min incubation).

Gergely *et al.* [2] also observed that the activation of glycogen synthase in unfiltered liver extracts from fructose-injected rats occurred without latency. Since gel filtration restored the sequential inactivation of phosphorylase and activation of glycogen synthase, the 'deinhibition' in the unfiltered extract was attributed to the presence of fructose 1-phosphate [2]. However, the latency is generally much less pronounced in unfiltered extracts from control animals, probably because of the post-mortem accumulation of substances like AMP and Mg^{2+} [5]. We have therefore compared the enzyme activity changes during incubation of unfiltered liver extracts from four fructose-injected and four glucagon-injected rats; the rate of inactivation of phosphorylase and the activation pattern of glycogen synthase were closely similar in the two conditions (results not shown). In a separate experiment fructose 1-phosphate was added to a control liver extract at concentrations (1–3 mM) that are expected to occur in a 20% (w/v) liver extract after fructose injection. No effects were observed at these concentrations.

We conclude that the accumulation of fructose 1-phosphate in the liver may at least in part explain a fructose-induced increase in the concentration of phosphorylase *a*. Other mechanisms may contribute as well [7,8]. The accumulation of fructose 1-phosphate does not explain, however, the fructose-induced activation of glycogen synthase that occurs in some experimental conditions. In general, the present data argue against the use of complex organic salts when the biological effects of metabolites are to be examined.

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Buffer capacity of intracellular Ca^{2+} indicators

The intracellular Ca^{2+} indicators designed by Tsien (1980) and Grynkiewicz *et al.* (1985) are now standard biochemical tools. Quin2, the first probe to become generally available and the most widely used, does involve the disadvantage of a substantial buffer capacity. Although there need be less buffering with more recent probes, reviewers still note the lack of attention to this point (DeLisle & Williams, 1986).

Some experiments with quin2 in synaptosomes illustrates a straightforward measurement of indicator buffer capacity. The synaptosomes were prepared from guinea-pig cerebral cortex (Gray & Whittaker, 1962; Hajos, 1975) and suspended in a Tris-buffered (pH 7.4) balanced salt solution containing 2 mM-$CaCl_2$. The ^3H_2O-permeable, [^{14}C]sucrose-impermeable space of synaptosomes prepared by the more convenient procedure of Hajos (1975), 4.2 ± 0.3 μl/mg of protein, mean $\pm$ S.E.M., $n = 6$, closely matched values for intrasynaptosomal volumes in the original preparation (4 μl/mg of protein at physiological tonicities; Marchbanks, 1975). Following quin2 loading (Ashley *et al.*, 1984) particulate suspensions of up to 350 μg of protein/ml were maintained at 37 °C and stirred continuously. Total intrasynaptosomal chelator concentrations were found by comparing the Ca^{2+}-saturated fluorescence of synaptosomes with Ca^{2+}-saturated free acid standards (conveniently prepared by mild alkaline hydrolysis of the ester). The ratio of Ca^{2+}-complexed to free quin2 ([CaQ]/[Q]) was also measured fluorimetrically (Tsien *et al.*, 1982) with the precautions noted by Ashley

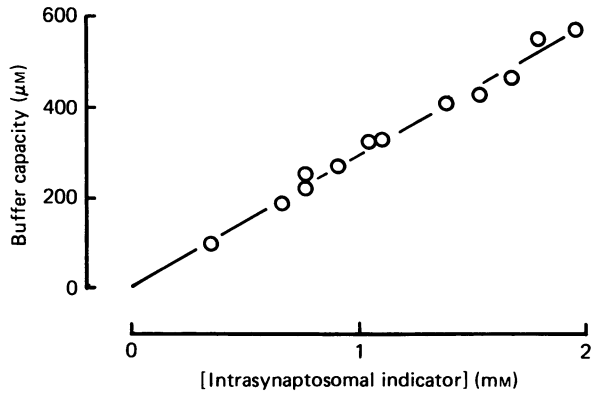

Fig. 1. Buffering by intrasynaptosomal quin2

The calculated practical buffer capacity of entrapped indicator for 10-fold increases in $[Ca^{2+}]_i$, B_{Ca} ($= 9[CaQ][Q]/10[CaQ]+[Q]$), is plotted versus [total trapped indicator]. [Q] and [CaQ] were obtained by solving the simultaneous equations involving total trapped indicator concentration, [CaQ+Q] (measured as described), and the ratio [CaQ]/[Q] (measured as described by Tsien *et al.*, 1982, and Ashley *et al.*, 1984). From the regression equation, $B_{Ca} = 290\ \mu M\text{-}Ca^{2+}/1$ mM-quin2. [For a K_d of 115 nM in cytoplasm (Tsien *et al.*, 1982) these data give a value for unstimulated $[Ca^{2+}]_i$ of 215 ± 5 nM, mean $\pm$ S.E.M., $n = 12$ preparations, in a medium containing 2 mM-$CaCl_2$.]

et al. (1984). The fluorimeter (Perkin–Elmer LS3) had a pulsed xenon source and no photobleaching occurred.

The practical buffer capacity represents the calcium bound by internal quin2 as $[Ca^{2+}]_i$ increases 10-fold from the baseline level. That is,

$$pCa - pCa' = 1 = \{pK_d + \log([Q]/[CaQ])\} - \{pK_d + \log([Q']/[CaQ'])\}$$

where K_d is the dissociation constant of CaQ (assuming 1:1 stoichiometry) and primed values occur after 10-fold increases in $[Ca^{2+}]_i$. This is of course a general equation for practical buffer capacity, and is simplified, with the elimination of K_d, in the legend to Fig. 1. (As usual, concentrations should be replaced by activities for precision.)

Fig. 1 illustrates a value for buffer capacity averaged from several preparations. At the relatively high concentrations needed for these measurements the entrapped indicator is an internal Ca^{2+}-buffer with a capacity similar to that of intraterminal mitochondria (Scott *et al.*, 1980). Its affinity for Ca^{2+} (K_d approx. 0.1 μM) is however close to that of cytosolic calcium-binding proteins and the endoplasmic reticulum, or indeed any mechanism buffering $[Ca^{2+}]_i$ near 0.2 μM. Rises in $[Ca^{2+}]_i$ (e.g. after depolarization) will grossly understate net Ca^{2+} uptake (overwhelmingly the increase in [CaQ], as long as no significant redistribution of internal cation occurs). During slow quin2 loading, cells and synaptosomes do seem to undergo net Ca^{2+} influx (Tsien *et al.*, 1982; Ashley *et al.*, 1984), but subsequent $[Ca^{2+}]_i$ transients will obviously be considerably distorted, or entirely lost.

The buffer capacity of quin2 should strictly limit its use even as an indicator for *equilibrium* free calcium measurements. Its (readily measurable) buffering could in fact be exploited deliberately to modify or abolish transients, looking for effects on stimulus–secretion and stimulus–response coupling.

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